Summary

Microorganisms live in nearly all natural environments were water and nutrients are available. They tend to attach strongly to all surfaces, like inorganic and organic living or dead materials. Once microorganisms are attached to a surface, a multi-step process starts resulting in the formation of a complex, adhering microbial community called a "biofilm". In a biofilm, different species and strains of microorganisms efficiently cooperate in order to protect themselves against environmental stresses and to facilitate more efficient nutrient uptake. Biofilms can be beneficial, like in the production of cheese or beer and purification of sewage and wastewater; moreover, they play an important role in several biological processes for the maintenance of health. However, most often biofilms produce severe detrimental effects in industry and cause serious health problems in humans and animals. As biofilms consist of microecosystems of different species they are hard to combat, and the increasing number of antibiotic-resistant bacterial strains indicates that a great deal of research effort should focus on the development of new non-antibiotic-based technologies to combat health-threatening biofilms.

Chapter 1 presents an overview of the interaction of suspending particles with air bubbles, which forms the basis of a separation process extensively used in industry, known as flotation. The principle of this technique is the creation of an upward flow of air bubbles that collide with suspended particles forming bubble-particles aggregates. Particles adhering to air bubbles have a lower density than the liquid phase and rise to the liquid surface forming froth. Finally, the froth on the surface can be mechanically separated from the liquid. While flotation processes have been used in industry for about hundred years and have been extensively studied, little interest has been paid to the ability of air bubbles to remove adhering particles or microorganisms from surfaces, although this technology seems to have promising applications in several industrial as well as biomedical fields. Therefore, the aim of this thesis is to study the mechanisms involved in air-bubble induced detachment of colloidal particles and microorganisms from surfaces, and the variables influencing this process.

The detachment of micron-sized polystyrene particles adhering to quartz surfaces by passing air bubbles in a parallel plate flow chamber has been studied in **Chapter 2**. A linear decrease of particle detachment with an increasing air bubble velocity was found. While up to 95% of adhering particles were detached by an air bubble moving at a velocity of about 2 mm s^{-1}, negligible detachment was achieved at velocities greater than 14 mm s^{-1}. Furthermore, a linear decrease in particle detachment was observed with a decrease in liquid-air interfacial tension, with different slopes for different air bubble velocities. Finally, particle detachment could be increased by the passage of multiple air bubbles.

After having concluded that moving air bubbles can be employed to detach micron-sized particles from collector surfaces, and that the detachment process can be optimized by adjusting the air bubble velocities, surface tensions, and the number of bubbles applied, other variables that could influence the detachment process were investigated. In **Chapter 3**, the detachment of polystyrene particles adhering to collector surfaces with different electrostatic charge and hydrophobicity by attachment to a passing air bubble was studied. Particle detachment decreased linearly with increasing air bubble velocity and decreasing liquid-vapor interfacial tension, regardless of the collector surface properties. However, particle detachment from hydrophilic, positively charged surfaces was most sensitive to variations in air bubble velocity and liquid-vapor interfacial tension, while detachment from hydrophobic, negatively charged surfaces was affected least. It was suggested that the influence of air bubble velocity predominantly occurs through the bubble/particle contact time, which needs to be long enough to allow sufficient thinning of the liquid film between the bubble and the particle for the detachment forces to become effective. On the hydrophobic collector surfaces, this thinning may be assisted by a nearly spontaneous de-wetting of the collector surface, therewith decreasing the influence of other factors, such as air bubble velocity or liquid-vapor interfacial tension. Upon multiple passages of air bubbles, approximately 80% of the particles could eventually be detached, regardless of the properties of the collector surfaces, illustrating that the probabilities of detachment by multiple air bubble passages are additive.

Subsequently, the influence of the particle properties in detachment induced by passing air bubbles was determined. Firstly, negatively and positively charged polystyrene particles, with the same size, adhering to the same collector surfaces utilized in Chapter 3, were used to study the effect of electrostatic interactions in the detachment process (**Chapter 4**). While electrostatic interactions between particles and collector surfaces were found to be

important, electrostatic interactions between adhering particles and passing air bubbles were found little or no relevant. Interestingly, detachment efficiencies up to 75% could be achieved even for positively charged particles adhering to a negatively charged collector surface, provided the velocity of the air bubble was low (2.37 mm s^{-1}) and the interfacial tension at the liquid-air interface was high (70.1 mJ m^2). The detachment process was more sensitive to air bubble velocity under conditions of electrostatic attraction between particle and collector surfaces than under conditions of electrostatic repulsion, but appeared equally sensitive to the liquid-air interfacial tension. Secondly, in **Chapter 5** particle size was found to be an important factor in air bubble induced detachment of colloidal particles from collector surfaces, as in general polystyrene particles with a diameter of 806 nm detached less than particles with a diameter of 1400 nm. Particle detachment increased linearly with decreasing air bubble velocity and increasing liquid-air interfacial tension, regardless of the particle diameter. Polystyrene particles with a diameter of 806 nm were less sensitive to the air bubble velocity than particles with a diameter of 1400 nm. However, the larger particles were less sensitive to variations in liquid-air interfacial tension than the smaller ones. Hence, it is proposed that for larger particles, the liquid film in between the air bubble and the particle takes more time to thin down and form the three-phase contact, necessary to induce a detachment force.

After that the mechanisms of air bubble-induced detachment of particles from surfaces were understood to a sufficient degree, research focused on the potential of this technique for detaching microorganisms from surfaces. In **Chapter 6**, an overview of the interactions between microorganisms and substratum surfaces is given. A distinction is made between adhesion, immobilization and retention of microorganisms on substrates, as it is believed that those three types of interaction forces mediate biofilm formation. A microorganism adheres to a substratum surface when it maintains the same separation distance from the substratum surface in the time, through a balance between Lifshitz-Van der Waals, electrostatic, Lewis acid-base and Brownian motion forces, homogeneously acting over the entire substratum surface. Consequently, adhering microorganisms are still free to move parallel to the substratum, as opposed to immobilization. Immobilized microorganisms adhere to the substratum but are kept at the same position due to lateral interaction forces, polymer bridging or chemical bonds. Finally, retention of adhering microorganisms denotes that

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microorganisms remain adhering on a substratum surfaces after application of an external force.

In **Chapter 7**, the detachment of bacteria (*Streptococcus sobrinus* HG1025, *Streptococcus oralis* J22, *Actinomyces naeslundii* T14V-J1, *Bacteroides fragilis* 793E, and *Pseudomonas aeruginosa* 974K) from hydrophilic glass and hydrophobic DDS-coated glass surfaces in the absence and presence of a conditioning film was analyzed. As for polystyrene particles (chapters 2 and 3), the detachment percentage increased when the velocity of the passing air bubble decreased, regardless of the bacterial strain and substratum surface hydrophobicity involved. However, the variation in percentage detachment by a passing air bubble depended greatly upon the strain and substratum surface involved. For instance, *P. aeruginosa* 974K did not detached from DDS-coated glass when the air bubble moved at high velocity (i.e. 13.6 mm s^{-1}), while for others strains detachment percentages between 80 and 90% were observed. At low air bubble velocities the hydrophobicity of the substratum had no influence on the detachment, but at high air bubble velocities all bacterial strains were more efficiently detached from hydrophilic glass. Furthermore, the presence of a conditioning film could either inhibit or stimulate detachment. Finally, the shape of the bacterial cell played a major role in detachment at high air bubble velocities, and spherical strains (i.e. streptococci) detached more efficiently than rod-shaped organisms.

In Chapter 1, the anchoring of adhering microorganisms to the substratum surface through the production of extracellular polymeric substances (EPS) it is emphasized as one of the important stages of biofilm formation on surfaces, how EPS provides mechanical stability to the biofilm, and how it protects the microorganisms in the biofilm against antimicrobials and the host immune system. In **Chapter 8**, the role of EPS in the initial adhesion and air bubble-induced detachment of *P. aeruginosa* to substratum surfaces with different hydrophobicity was investigated. For this purpose, two strains of *P. aeruginosa* are used: one EPS-producing strain (SG81) and a non-EPS-producing (SG81R1) strain. The release of EPS by SG81 was concurrent with a decrease in surface tension of a bacterial suspension from 70 to 45 mJ m^{-2}. Both strains adhered faster and in higher numbers to the hydrophilic than to the hydrophobic substratum, but the initial deposition rates and numbers of adhering bacteria in a stationary-end point were highest for the non-EPS-producing strain SG81R1, regardless of the substratum hydrophobicity. Both the EPS-producing as well as the non-EPS-producing strain adhered less to substrata pre-coated with isolated EPS of strain SG81. In contrast to an

anchoring effect of EPS, as expected and despite its EPS production, SG81 was detached by a passing air bubble in slightly higher percentages than the non-EPS-producing SG81R1. Detachment percentages were, however, low for both strains, possibly demonstrating that also the non-releasing strain releases minor, but at the level of the individual adhering organisms, sufficient amount of EPS to facilitate anchoring.

In a second experiment, also described in **Chapter 8** it was investigated whether bacteria detached by passing air-bubbles had left "footprints" behind with an influence on adhesion of newly depositing bacteria. Re-deposition on glass, from which adhering Pseudomonas had been detached by passing air-bubbles, was highest for non-EPS-producing SG81R1 and decreased linearly with the number of times these cycles of detachment and deposition were repeated to become similar to the re-deposition of SG81 after six cycles. This likely indicates that *P. aeruginosa* SG81 leaves the substratum surface nearly completely covered with EPS after detachment, therewith discouraging adhesion, while confirming that SG81R1 only releases minor amounts of surface active EPS, completely covering the substratum after repeated cycles of detachment and adhesion. The progressive coverage of glass by EPS after various cycles of detachment and deposition could be visualized by atomic force microscopy, showing a thick and irregular EPS layer (up to 32 nm) already after the first detachment cycle of EPS-producing strain SG81. The putatively non-EPS-producing strain SG81 R1 only left a 9 nm thick irregular layer after one cycle. X-ray photoelectron spectroscopy indicated that bacterial footprints left on glass after bacterial detachment, consisted of uronic acids, whose prevalence increased with the number of detachment and deposition cycles. Finally, it is concluded that the EPS released by *P. aeruginosa* SG81 discourages its adhesion to inert substrata.

A potential biomedical application of air bubble induced removal of microorganisms from surfaces is contemplated in the last section of this thesis. Bacterial adhesion and subsequent biofilm formation on human soft tissues with severe health consequences is encountered in many intra-abdominal diseases, including peritonitis. **Chapter 9** gives an overview of the causes of peritonitis, its different origins, the diversity and complexity of the biofilms found on the peritoneum, its health consequences, and the current treatment management of peritonitis. Antimicrobial therapy, elimination of the infection source by debridement and reconstruction of the gastrointestinal tract during laparatomy, followed by postoperative peritoneal lavage are the normal stages of peritonitis treatment. Ideally,

peritonitis should be cured with a single operation, but unfortunately infection often recurs and reoperations including repeated lavage of the peritoneal cavity have to be performed, increasing mortality risks. The high mortality and morbidity of patients suffering peritonitis (i.e. up to 60%), even after the development of new less invasive surgical techniques (i.e. laparoscopy), point to the need for developing new methods treatment.

One new technique for improving the treatment of peritonitis could be the use of air bubble-containing lavage liquids, in order to enhance the efficiency of the cleansing procedure of the peritoneal cavity. With this biomedical application in mind, an inventory of the hydrophobicities of the different peritoneal tissues in the living rat was made in **Chapter 10**. Peritoneal tissues were divided in mesentery, parietal and visceral peritoneum, and their hydrophobicity was determined by the sessile drop method. All peritoneal tissues were hydrophilic with water contact angles varying from 0 to 61 degrees. Mesentery and visceral peritoneum recovering the intestines were significantly more hydrophilic than parietal and other visceral peritoneal tissues. In general, visceral peritoneum was the most hydrophobic tissues, and visceral peritoneum covering the kidneys (61 degrees) and the stomach (54 degrees) was more hydrophobic than covering other organs, i.e. spleen (49 degrees), liver (45 degrees) and bladder (41 degrees). The hydrophilicity of the peritoneal tissues is probably due to surface-active phospholipids adsorbed on the serous membrane that give excellent lubricant properties, reducing wear and exfoliation of epithelial cells. In summary, peritoneal tissues involved in adsorptive and exchange functions and requiring lubrication are more hydrophilic than tissues with more important and protective functions.

In **Chapter 11**, the general discussion of this thesis, critical points in the application of air bubble-induced detachment in medicine or industry are discussed, as related to the observations made in a model system, i.e. the parallel plate flow chamber. It is pointed out that efficiency of biofilm detachment will greatly depend on the stage of biofilm formation. In the particular case of peritonitis, it is suggested that air bubble containing lavage liquids could improve the efficiency of peritoneal lavage, provided air bubbles generated in the peritoneal lavage liquids are stable (i.e. do not coalesce) and can be directed towards the infected tissue sites.